

J. Wei

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6: A23C 7/02		A1	(11) International Publication Number: WO 97/02753
			(43) International Publication Date: 30 January 1997 (30.01.97)
(21) International Application Number: PCT/DK96/00301		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 3 July 1996 (03.07.96)		Published With international search report.	
(30) Priority Data: 0819/95 1221/95 12 July 1995 (12.07.95) DK 2 November 1995 (02.11.95) DK			
(71) Applicant (for all designated States except US): NOVO NORDISK A/S (DK/DK); Novo Allé, DK-2880 Bagsvaerd (DK).			
(72) Inventor; and (75) Inventor/Applicant (for US only): OLSEN, Hans, Sejr (DK/DK); Novo Nordisk a/s, Novo Allé, DK-2880 Bagsvaerd (DK).			
(74) Common Representative: NOVO NORDISK A/S; Corporate Patents, Novo Allé, DK-2880 Bagsvaerd (DK).			
(54) Title: CLEANING-IN-PLACE WITH A SOLUTION CONTAINING A PROTEASE AND A LIPASE			
(57) Abstract A method of cleaning-in-place soiled process equipment comprising circulating a solution comprising a protease and a lipase for a sufficient period of time to permit action of the enzymes.			
<p>method for dairy or slaughter house comprising a <u>protease</u> and a <u>lipase</u></p> <p>Discussion text of C-I-P not at all</p>			

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Ghana	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Cote d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Switzerland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

CLEANING-IN-PLACE WITH A SOLUTION CONTAINING A PROTEASE AND A LIPASE

FIELD OF INVENTION

5

This invention relates to an enzymatic method of cleaning-in-place soiled process equipment, in particular dairy and slaughter house process equipment.

10 BACKGROUND OF THE INVENTION

Cleaning-in-place (CIP), which has replaced hand cleaning in, e.g., dairies, breweries and all potable liquid installations, involves circulating non-foaming or low foaming 15 detergents through process equipment in the assembled state.

A typical basic CIP sequence may consist of the following five stages (for reference see "Hygiene for Management" by Richard A. Sprenger, 5th Ed., p. 135, published by Highfield Publications):

- 20 (1) pre-rinse with cold water to remove gross soil;
(2) detergent circulation to remove residual adhering debris and scale;
(3) intermediate rinse with cold water to remove all traces of detergent;
25 (4) disinfectant circulation to destroy remaining microorganisms;
(5) final rinse with cold water to remove all traces of disinfectants.

The time allowed for each operation must be 30 determined for each particular plant or circuit being cleaned.

The detergent in step (2) in the above mentioned sequence is often 0.5-1% NaOH/KOH (+/- surfactants) at 75-85°C followed by a rinsing with water followed by a treatment with 35 0.5-1% HNO₃ (+/- surfactants) at 10-50°C. The surfactants used

are typically selected from nonionic and/or anionic surfactants often in combination with sequestering agents.

The industry wants more gentle cleaning media than the ones described above; a new cleaning media should offer one or more of the following advantages: Reduction of the water consumption, less damage to the equipment, lower temperatures, less risk for residues of surfactants and/or caustic and/or acids and/or sequestering agents in the food or beverage, less risk for accidents to the people handling the cleaning media. For membrane cleaning media also an improved cleaning efficacy is wanted.

SUMMARY OF THE INVENTION

15 In this invention it is surprisingly found that a solution comprising a protease and a lipase is very efficient in cleaning, e.g., process equipment containing residues of milk or burnt milk.

Accordingly, the present invention relates to a 20 method of cleaning-in-place soiled process equipment comprising circulating a solution comprising a protease and a lipase for a sufficient period of time to permit action of the enzymes.

25 DETAILED DISCLOSURE OF THE INVENTION

The method of the present invention may be applied to cleaning-in-place of any process equipment known in industry.

30 The method is particularly well suited for cleaning process equipment that prior to cleaning has contained materials containing proteins, fats or carbohydrates, in particular materials that prior to cleaning has contained fats and proteins such as milk, whey, cheese, cream, butter, milk 35 based desserts, fermented milk products such as yoghurt, ymer,

Gaio, meat, meat emulsions, sausages, whole meat cuts, feed products, liquid feed products, soy milk, tofu, fermented oriental fat-containing foods, extruded foods such as spaghetti and egg products, mayonnaise, sauces such as 5 bearnaise sauce, fish, fish emulsions, fish sausages and whole fish cuts.

The mechanism of the enzymatic cleaning of the hard surfaces of the process equipment is believed to be the following:

10 During enzymatic degradation of the soils (protein, fat, carbohydrates) a solubilization occur. Using a protease, the sections formed by the degradation of the protein become soluble. Using a lipase, the degraded fat becomes soluble at alkaline conditions. Using a carbohydrase, degraded polysac- 15 charides becomes soluble or the viscosity may be reduced significantly which help on the mechanical action needed for effective cleaning and rinsing.

Proteins are degraded to emulsifying or foaming products. When degraded by use of efficient serine proteases 20 the amphophilic properties of the peptides formed secure a high foam or emulsification effect. The peptides so formed also have a significant buffer capacity, and generally stabilize enzymes in solution.

Fats degraded by use of a lipase under alkaline 25 conditions form soaps or other amphipatic compounds. Using a 1.3 specific lipase monoglycerides are formed, which are known to be good emulsifiers.

When sufficient soil material is present for production of the above mentioned materials no, or very little 30 amounts of surfactants, other than those produced in situ during the cleaning is necessary, because the enzymes form soap and emulsifier from the degraded soil.

The following advantages with use of enzymes compared to traditional cleaning agents can be mentioned:

35

- In situ production of soap, emulsifiers, stabilizers due to the degradation of the soils.
 - Easier to rinse away.
 - Biodegradable waste products.
- 5 • Low foaming (especially an advantage in CIP, and particularly within membrane cleaning).
- Anticorrosive to metals and synthetic polymers used for membranes, sealings and tubes. Longer lifetime is found.
 - The time for cleaning may be reduced.
- 10 • The energy consumption may be reduced. (The enzymatic cleaning is performed at a lower temperature).
- The cleaning may be more efficient.
 - A possibility for phosphate free cleaning processes.
 - The waste water treatment may be cheaper.
- 15 • The waste water may be used for feed or (food).
- The waste water may also be used for other purposes like emulsifiers, buffers or cleaning agents for reuse or use in other places, such as lubrication purposes or polymer production.

20

The enzymatic cleaning according to the invention is effective, i.e. the substrate for the microorganisms (= the soil) is so effectively removed that growing of microbial cells is limited and/or inhibited. This is a very important
25 feature, in particular in the slaughter house and in the dairy industries, where the microbial control is very strict.

The method of the invention could therefore be very important in e.g. cleaning milking machines because it is a problem today to keep the inner surfaces of the milking
30 machines free of microorganisms.

As demonstrated in the enclosed examples the method of the invention works very well without any detergents being added. It may, however, in some cases be an advantage also to add a small amount of a surfactant, preferably a non-ionic
35 surfactant, in an amount of up to 1% w/w, preferably in an

amount of up to 0.1% w/w, more preferably in an amount of up to 0.025% w/w. Hereby, in some cases, an even better cleaning effect can be obtained, or the amount of enzymes can be reduced, or the cleaning time can be reduced.

5

Surfactants

If a surfactant is used it will normally be selected from the nonionic group or from the amphoterics. One or more of the following nonionic surfactants may be applied:

- 10 - glycerol derivatives,
 - sorbitan, glucose, sucrose derivatives,
 - fatty acid ethoxylates,
 - fatty acid ethoxylates propoxylates,
 - fatty alcohol ethoxylates,
- 15 - alkyl phenol ethoxylates,
 - fatty alcohol ethoxylates propoxylates,
 - fatty esters of polyalcohol ethoxylates,
 - end-blocked ethoxylates,
 - polypropylene glycols,
- 20 - polyethylene glycols.

Among the amphoterics one or more of following may be applied:

- alkylimidazoline,
- alkylbetaines,
- 25 - alkylamidobetaines,
- protein derivatives.

Process Equipment

According to the invention any process equipment
30 known in the art may be cleaned as described herein. In particular, all process equipment used in the food/feed industry may advantageously be cleaned as described in the present invention.

Also process equipment used for waste treatment,
35 e.g., oil/water separators, tanks, pipes, and membrane

separation equipment on, e.g., shipboard installations, in particular process equipment for the treatment of the so called "Gray water", may be cleaned as described in the present invention.

5 Dairy, slaughter house, brewery, feed, feed pelleting, fish and fish meal process equipment is particularly well suited.

Dairy and slaughter houses process equipment

10 In dairies the most difficult soil to remove is "burnt milk".

The milk forms gels on the inner surfaces (the surfaces that are in contact with the milk) of, e.g., heat exchangers, tanks, pipes, centrifuges, evaporators and
15 filters.

Also coagulated milk, melted and congealed cheese and milkstone, in particular all cheese manufacturing process equipment, may be problematic to clean. All these items may be effectively cleaned by the method of the present invention.

20 In slaughterhouses extruders, meat choppers and other equipment used in meat processing are difficult to clean. In meat and fish processing plants heat exchangers, cooking jars, coolers, storage tanks, pipes, centrifuges, evaporators, filters, sieves and hydrocyclones may be effectively
25 cleaned by the method of the present invention.

Milking machines

--- The use of enzymes for cleaning-in-place of milking machines are advantageous too. These machines are rather
30 difficult to clean as they consist of many "pockets", where soil can hide. There are many rubber and plastic tubes, which are sensitive to caustic, chlorine and acids.

Today milking machines are normally cleaned automatically by use of alkaline and/or chlorine based
35 surfactants together with sequestering agents. There is a wish

in the industry to reduce the amount of chemicals in this application as they can be difficult to rinse out completely.

By enzymatic cleaning the amount of chemicals may be reduced, the amount of rinsing water may be reduced, and the chance for residual amounts of surfactants in the milk is reduced.

Membrane processes

Membrane processes are widely used in many industries today. Reverse osmosis covering ultrafiltration, nanofiltration, hyperfiltration and microfiltration are techniques used in the dairy industry and in the fermentation industry (for production of products such as enzymes and pharmaceutical products).

15 The spiral wounded membrane types are in general not as alkali resistant as the plate and frame systems (dependent on the polymer type in question).

Also in the brewing industry a significant penetration of membrane processes for microfiltration is expected because of the wish to get rid of kieselgur filtration. Today microfiltration is not widely used due to fouling problems and to penetration of high molecular substances into the microfiltration membrane. The soil to be removed is presumably a build up of organic complexes of hop-resin, hop-oil, β -glucans, and tannic-protein products. By choosing the most suitable carbohydrases the method of the invention may give a solution to these problems.

Enzymes

30 According to the invention a cleaning solution containing a protease and a lipase is preferred, but depending on the soil in question the solution may also contain other enzymes such as carbohydrases.

The amount of enzymes used in the solution varies according to the type of enzyme and the soil in question. The

amount of each enzyme will typically be 0.00001-0.1% calculated as pure enzyme protein, preferably 0.001-0.01% calculated as pure enzyme protein.

Protease: Suitable proteases include those of 5 animal, vegetable or microbial origin. Microbial origin is preferred. Chemically or genetically modified mutants are included. The protease may be a serine protease, preferably an alkaline microbial protease or a trypsin-like protease. Examples of alkaline proteases are subtilisins, especially 10 those derived from *Bacillus*, e.g., subtilisin Novo, subtilisin Carlsberg, subtilisin 309, subtilisin 147 and subtilisin 168 (described in WO 89/06279).

Examples of trypsin-like proteases are trypsin (e.g. of porcine or bovine origin) and the *Fusarium* protease 15 described in WO 89/06270.

Examples of commercially available protease enzymes include Alcalase™, Savinase™, Esperase™ and Durazym™ products of Novo Nordisk A/S; Maxacal™, Maxapem™, Purafect™, and Purafect OXP™ products of Genencor International, and 20 Opticlean™ and Optimase™ by Solvay Enzymes.

Lipase: Suitable lipases include those of bacterial and fungal origin. Chemically or genetically modified mutants are included.

Examples of useful lipases include a *Humicola* 25 *lanuginosa* lipase, e.g., as described in EP 258 068 and EP 305 216, a *Rhizomucor miehei* lipase, e.g., as described in EP 238 023, a *Candida* lipase, such as a *C. antarctica* lipase, e.g., the *C. antarctica* lipase A or B described in EP 214 761, a *Pseudomonas* lipase such as a *P. alcaligenes* and *P.* 30 *pseudoalcaligenes* lipase, e.g., as described in EP 218 272, a *P. cepacia* lipase, e.g., as described in EP 331 376, a *Bacillus* lipase, e.g., a *B. subtilis* lipase (Dartois et al., (1993), *Biochemica et Biophysica acta* 1131, 253-260), a *B.*

stearothermophilus lipase (JP 64/744992) and a *B. pumilus* lipase (WO 91/16422).

Furthermore, a number of cloned lipases may be useful, including the *Penicillium camembertii* lipase described by Yamaguchi et al., (1991), Gene 103, 61-67), the *Geotricum candidum* lipase (Schimada, Y. et al., (1989), J. Biochem., 106, 383-388), and various *Rhizopus* lipases such as a *R. delemar* lipase (Hass, M.J et al., (1991), Gene 109, 117-113), a *R. niveus* lipase (Kugimiya et al., (1992), Biosci. Biotech. Biochem. 56, 716-719) and a *R. oryzae* lipase.

Examples of commercial lipases are Lipolase™, Lipolase Ultra™, Lipomax™ and Lumafast™.

Other types of lipolytic enzymes such as cutinases may also be useful, e.g., a cutinase derived from *Pseudomonas mendocina* as described in WO 88/09367, or a cutinase derived from *Fusarium solani pisi* (e.g. described in WO 90/09446).

A phospholipase may also be used; phospholipases may be obtained from porcine or bovine pancreas or from snake or bee venom, or they may be obtained from a microorganism. Examples of commercial phospholipases are Lecitase™ available from Novo Nordisk A/S and *Streptomyces chromofuscus* phospholipase available from Toya Jozo Co., Ltd.

Carbohydases: Depending on the polysaccharides in question to be removed one or more carbohydases such as amylases or cellulases may be used.

Amylase: Any amylase suitable for use in alkaline solutions can be used. Suitable amylases include those of bacterial and fungal origin. Chemically or genetically modified mutants are included. Amylases include, for example, α -amylases obtained from a special strain of *B. licheniformis*, described in more detail in British Patent Specification No. 1,296,839. Particularly preferred are Termamyl™ and Duramyl™, available from Novo Nordisk A/S.

Cellulase: Any cellulase suitable for use in alkaline solutions can be used. Suitable cellulases include those of bacterial and fungal origin. Chemically or genetically modified mutants are included. Suitable cellulases are disclosed in US 4,435,307. Particularly preferred is Celluzyme™ produced by a strain of *Humicola insolens*, available from Novo Nordisk A/S.

Cleaning-in-place

10 The method of the invention is particularly well suited for cleaning process equipment that prior to cleaning is soiled with a material containing proteins, fats or carbohydrates, in particular process equipment that prior to cleaning is soiled with a material containing fats and proteins.
15 teins.

 The solution containing the enzymes is circulated through the process equipment as known in the art. The solution may contain no surfactants other than those produced from fats and proteins either in situ and/or from an earlier
20 cleaning, or it may contain a small amount of a surfactant as described above.

 The time needed for effective cleaning depends on many factors such as the process unit to be cleaned, the kind of soil, the thickness and hardness of that soil, and the
25 temperature and pH of the solution containing the enzymes. However, a sufficient period of time will normally be from 10 minutes to 10 hours, preferably from 30 minutes to 3 hours; a sufficient temperature of the solution will typically be in the range of from 10°C to 90°C, preferably in the range of
30 from 20°C to 80°C, more preferably in the range of from 40°C to 80°C, a typical temperature will be around 50°C; and the pH of the solution will typically be above 7, preferably be in the range of from pH 8 to pH 10.

 A typical CIP-sequence according to the invention
35 may consist of the following steps:

I: Rinse with water - Enzymatic treatment - Rinse with water.

II: Rinse with water - Enzymatic treatment - Rinse with water
- Acid treatment - Rinse with water.

5

III: Rinse with water - Acid treatment - Rinse with water -
Enzymatic treatment - Rinse with water.

IV: Enzymatic treatment - Acid treatment - optionally rinse
10 with water.

V: Acid treatment - Enzymatic treatment - optionally rinse
with water.

15 VI: Enzymatic treatment - Rinse with water - Acid treatment -
optionally rinse with water.

VII: Acid treatment - Rinse with water - Enzymatic treatment -
optionally rinse with water.

20

Buffers

During enzymatic hydrolysis of protein- and fat-
containing material at pH > 7 carboxyl groups will be nearly
fully dissociated. This leads to a net release of H⁺ by
25 cleaving of peptide bonds and by cleaving of ester bonds in
triglyceride.

In order not to release small and volatile fatty
acids (with bad smell), e.g., butyric acid in milk-fat, the
pH-value is kept above 7, preferably above 8. Buffers with
30 high capacity and/or in high concentrations (e.g. > 0.1 M) may
be used, or it may be preferred to use a NaOH/KOH dosing as in
a pH-stat or it may be chosen to have a high pH-value from the
start of the cleaning and then let the pH drop from a high
value to a lower value during the cleaning.

Buffers which bind significant amounts of free Cations may reduce the hydrolytic activity of some proteases and some lipases.

Examples of useful buffer systems, which may be used according to the invention, are sodiumhydrogencarbonate (pH = 8) or sodiumcarbonate (pH = 8-10) in which the carbonate has a concentration below 0.05 M, preferably below 0.02 M. Also potassiumsodiumhydrogenphosphate at pH = 8 may be used at a concentration below 0.05 M, preferably at a concentration below 0.04 M.

The invention is further illustrated in the following examples which are not intended to be in any way limiting of the scope of the invention as claimed.

15

EXAMPLE 1

Total "Hydrolytic effect" (Model trials)

The total hydrolytic effect was measured as m
20 equivalents of NaOH/g of dry matter by use of pH-stat at 50°C, pH=8.0 on the basis of a 0.4% suspension of burnt whole milk powder. After addition of 0.025% Esperase 8.0 L (available from Novo Nordisk A/S) and/or 0.025% Lipolase 100 L (available from Novo Nordisk A/S) the hydrolysis lasted for 30 minutes
25 whereafter the amount of NaOH was measured. The data are presented in Table 1:

Table 1

Data for hydrolytic effect on burnt whole milk (120°C for 30
30 minutes):

35

Conc. of Esperase 8.0 L (% w/w)	Conc. of Lipolase 100 L (% w/w)	m eqv. NaOH/g of dry matter
5 0.025	0	0.23
0	0.025	0.12
0.025	0.025	0.55

10 It can be seen from Table 1 that there is a significant synergistic effect of combining the protease and the lipase.

EXAMPLE 2

15

CIP of Heat Exchanger Plates

In pilot plant a cleaning-in-place of a plate heat exchanger used for high pasteurization of whole milk for 6 hours was demonstrated. The heat exchanger had a 2-3 mm layer 20 of burnt milk. A circulation of a solution containing 0.1% Esperase 8.0 L and 0.1% Lipolase 100 L, 2.0 g NaOH/l and 6.8 g KH_2PO_4 /l (pH=8), 50°C for 2 hours was used (both enzymes available from Novo Nordisk A/S). After this treatment the exchanger was clean. No other detergents than those produced 25 *in situ* during the cleaning was applied. This proves that the enzymes form soap and emulsifier from the degraded soil. In this test no acid treatment following the enzyme treatment was necessary.

30 EXAMPLE 3

CIP of Heat Exchanger Plates

A complete CIP-programme was carried out on heat exchanger plates that were heavily soiled after high 35 pasteurization of whole milk for 6 hours. The heat exchanger was rinsed in 50°C hot water for 10 minutes. Hereafter an

enzyme treatment using 0.1% Esperase 8.0 L and 0.1% Lipolase 100 L, 2.0 g NaOH/l and 6.8 g KH_2PO_4 /l (pH=8), 50°C for 60 minutes was carried out (both enzymes available from Novo Nordisk A/S). A rinsing was carried out for 5 minutes using 50°C hot water. A 30 minutes treatment using 0.5% HNO_3 was made. Finally the heat exchanger plates were clean. It should be noted that in this Example an acid treatment was necessary due to a shorter enzyme treatment (60 minutes) compared with Example 2 in which the enzyme treatment lasted 2 hours.

10

EXAMPLE 4CIP of Heat Exchanger Plates

A complete CIP-programme was carried out on heat exchanger plates that were heavily soiled after high pasteurization of whole milk for 6 hours. The heat exchanger was rinsed in 50°C hot water for 10 minutes. Hereafter a treatment using 0.5% HNO_3 for 30 minutes at 50°C was made. The acid was rinsed out with water and an enzyme treatment using 0.1% Esperase 8.0 L and 0.1% Lipolase 100 L, 2.0 g NaOH/l and 6.8 g KH_2PO_4 /l (pH=8), 50°C for 60 minutes was carried out (both enzymes available from Novo Nordisk A/S). A final rinsing was carried out for 5 minutes using 50°C hot water. The heat exchanger was clean. 0.2%

25

The cleaning result achieved in Example 4 gave the same result as the cleaning result achieved in Example 3.

EXAMPLE 530 Milking machines

The aim of cleaning milking machines was that the hydrolytic effect of the enzymes (protease + lipase) should match that of alkali (NaOH).

We have seen a surprising significant synergistic effect of protease and lipase for the hydrolysis of whole

milk. The hydrolytic effect on a cost equivalent dosage of 0.025% Esperase 8.0 L + 0.025% Lipolase 100 L (both enzymes available from Novo Nordisk A/S) at pH=8 seems to be equivalent to 0.32 g NaOH/l (pH=11.2).

5

EXAMPLE 6CIP of Heat Exchanger Plates

A complete CIP-programme was carried out on heat exchanger plates that were heavily soiled after high pasteurization of raw unhomogenized whole milk for 6 hours. The heat exchanger was rinsed in 50°C hot water for 10 minutes. Hereafter an enzyme treatment using 0.1% Esperase 8.0 L and 0.1% Lipolase 100 L, + 0.025% Dobanol 25-7 from Shell 15 A/S and 0.025 M NaHCO₃ (pH=8), 50°C for 60 minutes was carried out (both enzymes available from Novo Nordisk A/S). A rinsing was carried out for 5 minutes using 50°C hot water. A 15 minutes treatment using 0.5% HNO₃ was made. Finally the heat exchanger plates were clean.

20

EXAMPLE 7Total "Hydrolytic effect"

The total hydrolytic effect was measured on 25 unhomogenized/pasteurized whole milk (Table 2) and on homogenized/pasteurized whole milk (Table 3) as m eqv NaOH/g of dry matter by use of pH-stat at 50°C, pH=8.0 on the basis of a 0.4% suspension of milk. After addition of Esperase 8.0 L and/or Lipolase 100 L (both enzymes available from Novo 30 Nordisk A/S) the hydrolysis lasted for 30 minutes whereafter the amount of NaOH was measured.

Table 2

Data for hydrolytic effect on unhomogenized whole milk, S = 35 0.4% of dry matter, pH 8.0:

Conc. of Esperase 8.0 L (% w/w)	Conc. of Lipolase 100 L (% w/w)	m eqv. NaOH/g of dry matter
5 0.025	0	0.24
0	0.025	0.07
0.025	0.025	0.84

10 Table 3

Data for hydrolytic effect on homogenized whole milk, S = 0.4% of dry matter, pH 8.0:

Conc. of Esperase 8.0 L (% w/w)	Conc. of Lipolase 100 L (% w/w)	m eqv. NaOH/g of dry matter
0.025	0	0.25
0	0.025	0.18
20 0.025	0.025	0.88

It was additionally tried to add different concentrations of SDS (Sodium-dodecylsulphate) to the enzyme solutions but there was no effect calculated as (m eqv. NaOH/g of dry matter) whether or not SDS was added.

Different proteases (Esperase 8.0 L, Alcalase 2.5 L and Savinase 16 L, all available from Novo Nordisk A/S) were also tested giving the following results:

30

Table 4

Data for hydrolytic effect on homogenized whole milk, S = 0.4% of dry matter, pH 8.0. Effect of different proteases:

35

Conc. of protease (% w/w)	Conc. of Lipolase (% w/w)	m eqv. NaOH/g of dry matter
5 0.025 (Esperase)	0.025	0.85
0.025 (Alcalase)	0.025	1.05
0.025 (Savinase)	0.025	0.95

10 It can be seen from Table 4 that all three proteases perform fine.

EXAMPLE 8

15 Viscosity measurements

The viscosity was measured on diluted solutions (0.4% and 0.8%) of unhomogenized milk by use of a Höebbler viscosimeter at 25°C. The milk was tested alone, after addition of 0.025% Esperase 8.0 L + 0.025% Lipolase 100 L, and
20 after addition of 2.5 g NaOH/l. The results are presented below:

Product:	Treatment:	Kinematic viscosity:
25 Milk (0.4% DM)		0.965 mPa X S
Milk (0.8% DM)		0.990 mPa X S
Milk (0.4% DM)	0.025% E*+L*	0.968 mPa X S
Milk (0.8% DM)	0.025% E*+L*	1.001 mPa X S
30 Milk (0.4% DM)	2.50 g NaOH/l	0.997 mPa X S
Milk (0.8% DM)	2.50 g NaOH/l	1.020 mPa X S
2.50 g NaOH/l		0.955 mPa X S

35

wherein E* means Esperase 8.0 L, and L* means Lipolase 100 L.

It can be seen from the results presented above that the enzyme containing solutions have a lower viscosity than the 5 solutions with NaOH. The rinsing after enzyme treatment may therefore be more efficient.

EXAMPLE 9

10 Ultrafiltration

A plate and frame module DDS type 10 having 336 cm² membrane type GR 61PP, DDS, was used for the trials. The nominal water flux was according to the data sheet: 250-350 l/m²/h at 20°C, 4 Bar. This is recalculated to 17°C and 3.1 Bar (Avg) corresponding to 175-250 l/m²/h.

On a new and clean membrane the water flux was measured at the following parameters: Temp. 17°C, P (avg.)= 3.1 Bar, corresponding to around 67 ml/min, which is equivalent to 120 l/m²/h +/- 10%. This flux should be obtained on a 20 membrane after cleaning.

In all cases the membranes were soiled by ultrafiltration of 2 litre whole milk at 50°C for 120 minutes to approximately 25% dry matter (refraktometer).

Trial no.	NaOH	Cleaning system I.		Temp. °C	Cleaning system II.
		<u>Esperase</u> 8.0 L	<u>Lipo-lase</u> 100 L		% HNO ₃
6		0.025%	0.025%	50	0.125
7		0.025%		50	0.125
8			0.025%	50	0.125
9	2.5 g/L			50	0.125
10	2.5 g/L			75	0.125

Procedure:

1. The membranes were soiled by ultrafiltration of 2 litre whole milk at 50°C for 120 minutes to approximately 25% dry matter (refraktometer) using an inlet pressure of 3.2 Bar and an outlet pressure of 3.0 Bar. The flow through the pump was 3.5-4 litre per minutes.
2. The recirculation vessel was rinsed with water at 50°C. When it was clean the water was flowed through the module at no back pressure. This secures maximal flow through the module. This rinsing was carried out for 5 minutes. Hereafter the flux and the temperature were measured. The corrigated flux was then calculated.
3. Water having a temperature of 50°C was added. Na₂CO₃ was then added to a concentration of 0.01 molar. pH was adjusted to 8.0 by use of 1 N HCl. Hereafter the cleaning agents (enzymes or NaOH) were added.
4. Recirculation was initiated. Also the permeate was recirculated to the vessel. Recirculation was carried out for 60 minutes at 50°C by low pressure (means maximal flow). The

flux and temperature were measured for control purposes during the cleaning operation.

5. A cleaning was now made with the HNO_3 solution mentioned in 5 the work plan. Recirculation was initiated. Also here the permeate was recirculated to the vessel. Recirculation was carried out for 5 minutes at 25°C by low pressure (means maximal flow). The flux and temperature were measured for control purposes during the cleaning operation.

10

6. Finally the content was rinsed out of the vessel and cold water was added. After 5 minutes recirculation the water flux was measured at an average pressure at 3.1 Bar and the corrigated flux was calculated. This flux was the finally 15 obtainable flux after the cleaning operation.

The results are shown below in Table 5 (next page).

It can be seen from Table 5 that the flux is only at the 20 starting level ($120 \text{ l/m}^2/\text{h} \pm 10\%$) after treatment with a protease + lipase solution (see trial no. 6 in Table 5).

Table 5

Trial no.	Final concentration, % DM after 120 min.	Flux before cleaning l/h/m ² , corr.	Cleaning system.	Flux after cleaning I l/h/m ² , corr.	Cleaning system.	Flux after cleaning II l/h/m ² , corr.
9	27.0	17.5	NaOH: 0.25%, (Maximal flow) 50°C, 60 min.	50.6	0.125% w/w HNO ₃ (Maximal flow)	46.9
10	30.5	15.6	NaOH: 0.25%, (Maximal flow) 75°C, 60 min.	61.2	0.125% w/w HNO ₃ (Maximal flow)	48.3
6	25.0	10.2	Lipolase: 0.025% Esperase: 0.025% (Maximal flow)	69.9	0.125% w/w HNO ₃ (Maximal flow)	108.5
7	29.30	13.5	Esperase: 0.025% (Maximal flow)	23.9	0.125% w/w HNO ₃ (Maximal flow)	47.5
8	20.2	11.8	Lipolase: 0.025% (Maximal flow)	18.9	0.125% w/w HNO ₃ (Maximal flow)	45.3

CLAIMS

1. A method of cleaning-in-place soiled process equipment comprising circulating a solution comprising a protease
5 and a lipase for a sufficient period of time to permit action of the enzymes.

2. A method according to claim 1, wherein the process equipment is selected from the group consisting of heat
10 exchangers, tanks, pipes, centrifuges, evaporators, filters, extruders, meat choppers, cooking jars, coolers, storage tanks, sieves, hydroclones, ultrafiltration units, nanofiltration units, hyperfiltration units, microfiltration units and milking machines.

15

3. A method according to claim 1, wherein the process equipment is a dairy or a slaughter house process equipment.

4. A method according to claim 1, wherein the process
20 equipment prior to cleaning is soiled with a material containing fats and proteins.

5. A method according to claim 1, wherein the protease is a serine protease.

25

6. A method according to claim 5, wherein the protease is a subtilisin, preferably obtainable from *Bacillus*, most preferably subtilisin Novo, subtilisin Carlsberg, BPN', subtilisin 309, subtilisin 147 or subtilisin 168.

30

7. A method according to claim 5, wherein the protease is trypsin or the protease is obtainable from *Fusarium*.

23

8. A method according to any preceding claim, wherein the amount of protease in the solution is 0.00001-0.1% calculated as pure enzyme protein.

5 9. A method according to claim 1, wherein the lipase is of microbial origin, preferably obtainable from *Humicola*, *Mucor* or *Pseudomonas*.

10 10. A method according to any preceding claim, wherein the amount of lipase in the solution is 0.00001-0.1% calculated as pure enzyme protein.

15 11. A method according to claim 1, wherein the solution additionally contains a carbohydrase.

12. A method according to any preceding claim, wherein the amount of carbohydrase in the solution is 0.00001-0.1% calculated as pure enzyme protein.

20 13. A method according to claim 1, wherein the solution additionally comprises a surfactant in an amount of up to 1% w/w.

25 14. A method according to claim 1, wherein a sufficient period of time is from 10 minutes to 10 hours, preferably from 30 minutes to 3 hours.

30 15. A method according to claim 1, wherein the solution has a pH above 7, preferably a pH in the range of from pH 8 to pH 10.

16. A method according to claim 1, wherein the solution has a temperature in the range of from 10°C to 90°C.

17. A method according to claim 1, whereby growing of microbial cells in said process equipment is limited and/or inhibited.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 96/00301

A. CLASSIFICATION OF SUBJECT MATTER		
IPC6: A23C 7/02 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
IPC6: A23C		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
SE,DK,FI,NO classes as above		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
MEDLINE, BIOSIS, EMBASE, CLAIMS, WPIL, JAPIO, FOOD SCI.& TECHABS, FOODLINE		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 617585 C (HENKEL & CIE G.M.B.H.), 22 August 1935 (22.08.35), column 2, line 47 - line 64; column 3, line 1 - line 15	1-17
A	Från mjölk till Mejeriprodukter, Hygien, Livsmedelsbranchernas yrkesnämnd, Brevskolan, 1980, see especially page 26	1-17
A	WO 9423004 A1 (BASF AKTIENGESELLSCHAFT), 13 October 1994 (13.10.94)	1-17
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principles or theory underlying the invention "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
16 October 1996		17 -10- 1996
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86		Authorized officer Ake Lindberg Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

01/10/96

International application No.

PCT/DK 96/00301

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE-C- 617585	22/08/35	NONE	
WO-A1- 9423004	13/10/94	DE-A- 4310995 EP-A- 0692015	06/10/94 17/01/96

Form PCT/ISA/210 (patent family annex) (July 1992)

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.